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HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

FINAL DATA SUMMARY

for

SPENT PULPING LIQUOR

CAS No. 66071-92-9

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The American Forest & Paper Association
HPV Work Group

Consortium Registration #

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Spent Pulping Liquor Final Data Summary

AF&PA sponsored the HPV chemical, “sulfite liquors and cooking liquors, spent” (CAS No. 66071-92-9) (commonly referred to as “spent pulping liquor”). Spent pulping liquor is a byproduct of processing (or “cooking”) wood chips to remove the wood pulp to manufacture paper. The vast majority of spent pulping liquor is recycled for chemical or energy recovery at the production site. Spent pulping liquor may be traded between mills for these uses. A limited number of facilities transfer spent pulping liquor off-site for use in other processes. Exposure to humans or the environment is not expected to occur, except in cases of accidental release.

Spent pulping liquor is a corrosive complex mixture with a pH ranging from approximately 11.5 to 13.5. Spent pulping liquor is classified as a corrosive liquid under Department of Transportation regulations and labeled as such.

The composition of the complex mixture known as spent pulping liquor is highly variable, depending on several factors. These factors include the process and wood species used to manufacture the wood pulp, the type of end product for which the pulp is intended, the composition of the cooking liquor, and the type of digester equipment used in pulping. After reviewing volume, commercial use, and potential for human and environmental exposure from each pulping process, strong black liquor from an elemental-chlorine-free kraft process using a mixture of hardwood and softwood was selected as the most appropriate test material. More detailed justification for selection of this material is presented in the test plan.

While information is available about the chemistry and certain characteristics of spent pulping liquor, including its pH, there were essentially no data available on the required HPV/SIDS endpoints for spent pulping liquor. The lack of test data is not surprising in view of the corrosiveness of spent pulping liquor and that it is a byproduct that is almost always recycled at the production site and has only limited commercial use. Moreover, there is no analytical method for characterizing spent pulping liquor given that it is a complex mixture of hundreds of different constituents.

Although the likelihood of human or environmental exposure to spent pulping liquor is extremely low, AF&PA carefully reviewed the HPV test battery to determine whether these tests can and should be performed. The final summary document explains in detail the following conclusions:

Physicochemical Properties

Certain physical and chemical property tests (water solubility, octanol-water partitioning coefficient, and adsorption/desorption to soil) were designed for single pure materials and will not yield valid results given the complexity of the spent pulping liquor mixture. Hence, it is impossible to obtain a single value representing the nature of the mixture from a range of values from the many constituents of this mixture. These tests were not performed. In addition, none of these endpoints can be measured since an analytical method for spent pulping liquor is not available. While determining the boiling point and vapor pressure of spent pulping liquor was originally proposed, in commenting on the Test Plan, EPA suggested that this was not necessary; consequently, these endpoints were not determined. Determination of the melting point is not applicable because the material is a liquid.

Environmental Fate

Testing for photodegradation requires measurement of the degradation products of the test material. Hydrolysis as a function of pH is designed to test pure substances at environmentally relevant pH levels and does not apply to spent pulping liquor, a highly alkaline, complex mixture. Neither of these tests could be undertaken because an analytical method for spent pulping liquor is not available. Fugacity modeling is inappropriate for complex Class 2 substances such as spent pulping liquors, because the required inputs are either not available or are impossible to determine.

With respect to biodegradation, because the test protocol permits neutralization to pH levels compatible with survival of bacterial cultures, this test was undertaken. However, it is likely that neutralization altered the composition of the material. Using OECD test method 302B, spent pulping liquor was not found to be readily biodegradable with a level of 49.5% biodegradation by Day 28. However, a substantial level of inherent biodegradation was observed based on dissolved organic carbon depletion.

Ecotoxicity

Following neutralization of the test material, tests of aquatic toxicity to fish daphnia and algae were undertaken. For fish, the 96 hr LL_{50} was > 1000 mg/l with a No Observed Effect Loading Rate ($NOEL_r$) of 1000 mg/l. For daphnia, the 48 hr EL_{50} was > 1000 mg/l with a No Observed Effect Loading Rate ($NOEL_r$) of 1000 mg/l. For algae, the 72 hr E_rC_{50} (Average specific growth rate) was estimated as 142.98 mg/l; the 72 h E_bC_{50} (AUC) was estimated as 312.44 mg/l with a corresponding No Observed Effect Loading Rate ($NOEL_r$) of 5 mg/l for AUC and Average Specific Growth Rate.

Health Effects

Spent pulping liquor is very alkaline in nature, with a pH ranging from 11.5 to 13.5. It is known that exposure to highly corrosive materials induces pain in both humans and animals. Corrosive materials can cause severe ulcerations or necrosis (cell death) at the point of contact, i.e., the esophagus or gastric mucosa (stomach lining) when the test material is administered orally.

OECD and EPA guidelines provide that testing that causes pain in laboratory animals as a result of corrosive effects should not be undertaken; the Animal Welfare Act similarly proscribes testing that inflicts pain on animals. Dilution of spent pulping liquor in order to perform the tests would alter the fundamental nature of the test material, as different constituents precipitate out of the solution at different pH levels. Accordingly, mammalian testing with spent pulping liquor was not conducted.

The results of any mammalian testing would be related to the well established, but non-specific corrosive effects of high pH compounds. Ample data exist on other compounds to document the effects of high pH on humans and animals. For this reason, mammalian testing of spent pulping liquor was unnecessary.

In vitro genotoxicity testing on spent pulping liquor was conducted in bacteria (*S. Thyphimurium)* and mammalian cells [Chinese Hamster Ovary (CHO)]. In *Salmonella* no increases in mutation frequency were reported at any concentration with or without metabolic activation. In CHO cells, spent pulping liquor was clastogenic with S9 mix at 2500 ug/ml and without S9 mix at 5000 ug/ml; both concentrations were overtly toxic to the cells.

Overall Hazard Evaluation and Potential Exposure

For potential human health effects, the data on spent pulping liquor indicate that it is toxic (i.e., corrosive) due to its pH of 11.5 to13.5. Genotoxicity test results show no evidence of mutagenicity in *Salmonella* (i.e., Ames test) for spent pulping liquor. Chromosomal aberrations in Chinese hamster ovary (CHO) cells were evident only at concentrations of spent pulping liquor that were overtly toxic to the cells. Consequently, the only adverse health consequence that would be associated with any anticipated exposures to spent pulping liquor would be due to its corrosive nature. For potential ecotoxicological effects, the data on spent pulping liquor demonstrate that following neutralization (which would occur following dilution in the event of a spill) this substance would be non-toxic to aquatic organisms including fish and daphnia, and minimally toxic toward algae.

With respect to potential exposure to spent pulping liquor, it is important to note that it is a byproduct that is almost always recycled at the production site and has only limited commercial use. Almost all of the spent pulping liquor generated is recycled for chemical and energy recovery. Controlled management of the material minimizes any potential for human or environmental exposure. An extremely small percentage of spent pulping liquor is transferred via pipeline on site to adjacent plants for lignin extraction and dimethyl sulfide extraction. Remaining spent pulping liquor is then returned to the pulp mill to be recycled for chemical and energy recovery. Strong black liquor (i.e., spent pulping liquor with some water removed) may be transported by truck to other pulping facilities that use it for chemical and energy recovery. In the event of an accidental spill into a body of water, pH effects are likely until sufficient dilution had occurred.

List of AF&PA HPV Consortium Members

The American Forest & Paper Association HPV Consortium includes the following companies:

Abitibi Consolidated
Alliance Forest Products
Blue Ridge Paper Products
Boise Cascade Corporation
Bowater, Inc.
Buckeye Technologies, Inc.
Eastern Pulp and Paper, Inc.
Finch, Pruyn & Company, Inc.
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Greif Bros. Corporation
Gulf States Paper Corporation
Inland Paperboard and Packaging Company
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St. Laurent Paperboard Inc.
Stora Enso North America
Westvaco Corporation
Weyerhaeuser Company
Willamette Industries, Inc.

Spent Pulping Liquor

Final Data Summary

I. Description of Spent Pulping Liquor and Rationale for Selection of Test Material

AF&PA sponsored the HPV chemical, "sulfite liquors and cooking liquors, spent" (CAS No. 66071-92-9). This CAS number, also referred to as "spent pulping liquors," includes the liquors resulting from a variety of pulping processes within the pulp and paper industry.[1] Such processes include kraft, soda, sulfite, semichemical, and chemi-thermomechanical.

Almost all of the spent pulping liquor generated by any of these processes is recycled for chemical and energy recovery. Controlled management of the material minimizes any potential for human or environmental exposure from these activities.

Spent pulping liquor is variable in its composition, depending upon the wood species used to manufacture the wood pulp, the composition of the cooking liquor, and the type of digester equipment used in pulping, and the type of end product for which the pulp is intended. Using Agency guidance, AF&PA selected strong black liquor from the kraft process at an elemental-chlorine-free mill using a mixture of hardwood and softwood as its test material for the HPV program, as described further below.

EPA guidance suggests that testing the substance produced at the highest volume would be appropriate. (EPA Guidance, *Guidance for "What to Test" for the HPV Challenge*, draft dated 2/08/99.) Agency guidance also suggests that the selected test material should be "representative" of the sponsored CAS number. Strong black liquor from a bleached, elemental-chlorine-free (ECF) kraft process using a mixture of hardwood and softwood is most representative of spent pulping liquor in the pulping industry for several reasons:

> ***Volume*** – The kraft pulping process is by far the most significant process. Annual pulp production capacity from the kraft process is over 57 million tons.

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[1] Spent pulping liquors are defined as:

> the aqueous solution resulting from the reaction of lignocellulosic substances (wood or other agricultural fiber sources) with one or more pulping chemicals including those used in the kraft, sulfite, semichemical or other pulping processes. Composition is highly variable and includes excess pulping chemicals, dissolved and degraded cellulose, hemicellulose and lignin.

TSCA Chemical Substance Inventory, 1990 Supp., App. A.

Annual pulp production for the other processes fall well below this, in the range of 1-5 million tons per year. Consequently, there is more spent liquor generated by the kraft process than by all other processes combined.

Potential for Human/Environmental Exposure – Virtually all spent pulping liquor of any kind is recycled for chemical and energy recovery. The vast majority thus remains on-site, with two very minor exceptions.

- An extremely small percentage of spent pulping liquor is transferred via pipeline to an adjacent plant for lignin extraction and dimethyl sulfide extraction. Remaining spent pulping liquor is then returned to the pulp mill to be recycled for chemical and energy recovery.

- Strong black liquor may be transported by truck to other pulping facilities that use it for chemical and energy recovery.

While potential for exposure to spent pulping liquors is minimal, accidental release during transportation represents the most plausible source of environmental exposure. (When black liquor is transported, some water is typically removed, and the material is therefore referred to as "strong black liquor." Strong black liquor has a higher pH than weak black liquor, and thus would be expected to be on the higher end of the range given in Material Safety Data Sheets for black liquor.)

Kraft black liquor from a pulping process using a mixed blend of hardwood and softwood, and elemental chlorine free (ECF) bleaching is most representative of current industry practices. Most mills produce both softwood and hardwood pulp at a single location. Spent pulping liquor most often exists as a blend from processing both hardwood and softwood. In addition, United States production of bleached pulp is greater than unbleached pulp, and all bleached chemical pulp mills will be ECF as a result of the EPA Cluster Rule (recent air and water regulations specific to the pulp and paper industry).

Accordingly, the material that was used for testing of spent pulping liquor was spent strong black liquor taken from an ECF bleached kraft mill that used a mixture of hardwood and softwood. This provided the most appropriate material to represent CAS No. 66071-92-9, spent pulping liquor.

II. Chemical Composition of Spent Pulping Liquor

A. Kraft Black Liquor Composition

Strong black liquor contains between 50 and 70% solids, with the remainder being water. The solids are comprised of a complex mixture of both inorganic and organic constituents.

1. Inorganic Constituents

The inorganic constituents in black liquor are derived from the cooking liquor which is used to pulp the wood chips, and are comprised of sodium hydroxide (NaOH), sodium sulfide (Na_2S), sodium carbonate (Na_2CO_3), sodium sulfate (Na_2SO_4), sodium thiosulfate, ($Na_2S_2O_3$), and sodium chloride (NaCl). Collectively, inorganic salts constitute between 18 and 25% of the solids in black liquor.

2. Organic Constituents

The organic compounds found in black liquor are derived from wood. They are either 1) natural wood extractives (or their reaction products) that are released as a result of the pulping process, or 2) materials formed through the reactions of the pulping liquors with the lignin or cellulose components of wood. Therefore, the compounds can be classified as lignin derived, cellulose derived, or extractives derived. Typical content ranges in kraft liquor are:

<u>Lignin derived</u> (39-54%; primarily consisting of polyaromatic macromolecules with lesser amounts of low molecular weight alcohols, aldehydes and simple phenolic compounds such as phenol, p-methyl phenol, catechol and guaiacol),

<u>Cellulose derived</u> (25-35%; primarily a mixture of carboxylic acids such as formic, acetic, glycolic, lactic and glucoisosacchararinic), and

<u>Extractive derived</u> (3-5%; primarily resin acids and fatty acids which are converted to salts at the high Ph of the mixture).

In sum, spent pulping liquor can have hundreds of consituents.

B. Acid-Base Properties of Black Liquor

Black liquor is distinctly alkaline (caustic), with pH ranging from 11.5 to 13.5. Due to the presence of three distinct buffer systems, black liquor is highly buffered. These buffer systems, and their Pk_a values (representing their potential for dissociation) are:

Sulfide buffer: $Na_2S + H_2O \rightarrow NaHS + NaOH$ $\quad Pk_a \cong 13\text{-}13.5$

Phenolic buffer: $R\text{-}OH + NaOH \rightarrow R\text{-}ONa + H_2O$ $\quad Pk_a \cong 9.4\text{-}10.8$

Carbonate buffer: $Na_2CO_2 + H_2O \rightarrow NaHCO_3 + NaOH$ $\quad Pk_a \cong 10.2$

The high alkalinity is largely responsible for solubilizing the various organic constituents. If the pH is reduced, various organic constituents will precipitate, beginning with the components with low Pk_a values (e.g. the phenolics) and eventually those with higher Pk_a values (e.g. the carboxylic acids). Thus, the soluble component would vary as pH is reduced. Consequently, when the pH was adjusted in order to perform certain tests, the nature and composition of the test material was necessarily changed.

C. Analytical Complexity

There is no analytical methodology available to characterize the composition of spent pulping liquor. As noted above, spent pulping liquor is a mixture of numerous known, tentatively identified and unidentified components, and thus only some components would be available for calibration purposes. Given the complexity of the mixture, it is not currently possible to characterize black liquor as necessary to undertake a number of the SIDS endpoints. The HPV program does not encompass the kind of research program that would be necessary in order to develop an appropriate analytical method with sufficient sensitivity, if indeed it is even possible to do so. Consequently, because of the lack of an appropriate analytical methodology and the practical impossibility of developing such a method, many of the required SIDS endpoints that are part of the HPV Challenge program cannot be undertaken.

In commenting on the spent pulping liquor test plan, EPA requested that AF&PA characterize the neutralized test material to the extent possible at termination of the ecotoxicity testing. Analysis of such a complex material presents a difficult challenge. A Good Laboratory Practices-validated method for the analysis does not exist, and it is uncertain that the analytical results will be informative. However, EPA recognized these difficulties, qualifying its request for characterization "to the extent possible." While a research program to develop analytical methods was beyond the scope of the HPV program, AF&PA requested its technical arm, the National Council for Air and Stream Improvement (NCASI), to attempt an analysis of neutralized material. The results of this analysis on a sample of spent pulping liquor neutralized in the same manner as used to prepare samples for ecotoxicity testing is presented below.

D. Chemical Characterization of Spent Pulping Liquor

A split sample of the spent pulping liquor used for ecotoxicity testing was sent to the NCASI West Coast Regional Center for chemical characterization. The chemical characterization was performed on a dilution equivalent to the least dilute sample used for ecotoxicity testing (e.g., 1/1000 by weight). The analytes and analytical methods were selected based on techniques used by NCASI to chemically characterize pulp and paper industry wastewaters used for bioassay testing or for ecological assessment studies. These included non-chlorinated resin and fatty acids (by NCASI Method RA/FA 85.02) and phytosterols (by NCASI Method STER-97).

The spent pulping liquor sample was brought to room temperature and shaken prior to removing an aliquot for analysis. The required sample aliquot was weighed and then diluted with reagent water. The non-chlorinated resin and fatty acids were analyzed by NCASI Method RA/FA 85.02. Due to the concentrations measured, an additional 10 fold dilution was performed to bring the analytes within the calibration range. The NCASI method for phytosterols had not previously been applied to spent pulping liquors (and thus is not fully validated for that matrix), so the sample was analyzed using the GC/MS confirmatory technique described in NCASI Method STER-97. (Note: subsequent to the publication of STER-97, NCASI determined that N-methyl-N-trimethylsilyl-trifluoroacetamide [MSTFA] was a better derivatizing agent than the BSTFA originally specified in the method. Thus, MSTFA was used in these analyses.) For each analysis, a laboratory blank, a sample duplicate, and a sample matrix spike were analyzed for quality assurance.

Table 1 summarizes the resin and fatty acid analysis results. Although not included in the summary table, there were no detectable analytes in the method blank. All surrogate and matrix spike recoveries were within method specifications.

Table 2 summarizes the phytosterol analysis results. There were no detectable analytes in the laboratory blank. As noted, this method has not been fully validated on spent pulping liquors; therefore there are no applicable QC specifications. The surrogate recoveries are within the range previously observed for treated effluents and influents to treatment systems. The matrix spike recoveries for all analytes except β-sitosterol were within the ranges previously observed for influent analyses. Because the method has not been fully validated for spent pulping liquors, the method detection limit has not been determined for this matrix. For reference, the lower calibration limit for these analyses were 0.85 μg/L for campesterol, 1.22 μg/L for stigmasterol, 1.15 μg/L for β-sitosterol, and 1.30 μg/L for stigmastanol.

Table 1. Summary of Resin and Fatty Acid Analyses

	Sample	Sample Duplicate[1]	Average Concentration	Relative Percent Difference	Matrix Spike Concentration Measured
	µg/L	µg/L	µg/L	%	µg/L
Analytes					
Linoleic acid	16.3	14.6	15.4	11.0	137
Oleic acid	11.4	10.4	10.9	9.2	149
Pimaric acid	7.8	7.4	7.6	5.3	115
Sandracopimaric acid	1.9	1.6	1.5	20.0	112
Isopimaric acid	6.9	6.6	6.8	4.4	116
Palustric acid	11.2	14.0	12.6	22.2	132
Dehydroabietic acid	32.9	25.0	29.0	27.2	145
Abietic acid	37.5	42.5	40.0	12.5	157
Neoabietic acid	11.0	11.1	11.0	0.9	133
Surrogate Recoveries	% Recovery	% Recovery			% Recovery
Heptadecanoic acid	101	97			97
O-Methyl podocarpic acid	89	91			91

[1] Average of duplicate analyses of the same dilution of spent pulping liquor and background data for matrix spike analysis

Table 2. Summary of Phytosterol Analyses

	Sample Concentration	Sample Duplicate Concentration	Average Concentration	Relative Percent Difference	Matrix Spike Concentration Measured
	μg/L	μg/L	μg/L	%	μg/L
Analytes					
Campesterol	ND	ND	NA	NA	17.0
Stigmasterol	ND	ND	NA	NA	29.9
β-Sitosterol	6.9	5.1	6.0	29.9	26.0
Stigmastanol	ND	ND	NA	NA	28.9
Surrogate Recovery	% Recovery	% Recovery			% Recovery
Cholesterol	54.7	46.1			44.6

ND not detected
NA not applicable

III. Summary of Available Data

A. Physicochemical Data

The basic physicochemical data required in the SIDS battery includes melting point, boiling point, vapor pressure, partition coefficient (K_{ow}), and water solubility.

Class 2 substances such as spent pulping liquor are composed of a complex mixture of substances and are often difficult to characterize. Their composition is variable and cannot be represented by a definite chemical structural diagram. Due to this "multi-component" characteristic of spent pulping liquor, most physical property measurements are not appropriate as explained below.

Most of the required physicochemical properties tests in the SIDS battery are designed for a single, pure chemical. Due to the fact that spent pulping liquor is an extremely complex mixture of inorganic and organic constituents, many of the common physicochemical parameters are inapplicable.

The problem is further exacerbated by the lack of a suitable analytical procedure to measure spent pulping liquor. Absent a suitable analytical method for measuring the spent pulping liquor, some of the tests cannot be performed. For these reasons, many of the SIDS physicochemical tests in this category could not be performed or would not produce useful information. Tests that are inappropriate for this material include water solubility, octanol-water partitioning coefficient (K_{ow}), and adsorption/desorption to soil. At the recommendation of EPA, determination of the boiling point and vapor pressure of spent pulping liquor were not undertaken. Data on pH of spent pulping liquor are already available, and determination of the melting point is not necessary since the material is a liquid. The following narrative explains the above rationale in more detail.

1. Water Solubility

Spent pulping liquor is a complex mixture of inorganic and organic salts suspended or dissolved in water. A test for water solubility could be performed on the test material, but it would result in multiple values for individual constituents. Due to the lack of a suitable analytical method for the complex mixture, it is not feasible to measure the water solubility for the mixture.

As noted above, strong black liquor contains 50-70% solids. At solids contents below 50%, the inorganic salts contained in spent pulping liquor are completely dissolved in the aqueous portion of the liquor. Often, the 50% solids point (the point where the salts start precipitating) is referred to as the "solubility limit." At solids levels greater than 75%, Burkeite ($2Na_2So_4$ - Na_2CO_3) is the only salt that precipitates. Thus, between 50 to 75% solids, spent pulping liquor is essentially a water/organic-inorganic suspension (Adams et al. 1997).

2. Melting Point and Boiling Point

Because spent pulping liquor is a liquid under normal conditions, it is not necessary to determine the melting point. As previously noted, the EPA suggested that determination of the boiling point would provide little useful information and consequently this endpoint was not measured.

3. Octanol:Water Partition Coefficient (K_{ow})

Given the numerous organic and inorganic constituents in kraft black liquor, any assay used to estimate the partitioning properties would yield a range of values reflecting this complex mixture. Such values would be meaningless and would provide little, if any, useful information concerning the material. Consequently, the K_{ow} was not conducted on this mixture. In addition, the lack of an analytical method also precluded conducting this test.

4. pH

Already available data show that the pH of kraft black liquor ranges from 11.5 to 13.5 (various company Material Safety Data Sheets).

B. Environmental Fate Data

The fate or behavior of a chemical in the environment is determined by the rates or half-lives for the most important transformation (degradation) processes. The basic environmental fate data covered by the HPV Program includes biodegradation, stability in water (hydrolysis as a function of pH), photodegradation and transport and distribution between environmental compartments. Due to the complexity of spent pulping liquor and the lack of an analytical method, with the exception of biodegradation, none of these endpoints was determined. The rationale for this decision is described below.

1. Photodegradation

The practicability of performing this test is hindered by the lack of an analytical procedure to measure spent pulping liquor. A test of photodegradation cannot be performed, since the composition and quantity of the test material before and after exposure to sunlight cannot be measured.

2. Hydrolysis

With respect to the hydrolysis test, the required test (OECD 111) is designed to measure hydrolysis (stability in water) of pure compounds at several pH levels (4-9) that are likely to be found in the environment. Thus, the test is not applicable to the alkaline, complex mixture of spent pulping liquor. In addition, this endpoint could not be measured since an analytical method for spent pulping liquor was not available.

3. Biodegradation

Following neutralization, biodegradation testing of spent pulping liquor was undertaken using OECD test method Test Method 302 B, *"Zahn Wellens Test"* Spent pulping liquor was not found to be readily biodegradable. However, a substantial level of inherent biodegradation was observed based on dissolved organic carbon (DOC) depletion. Spent pulping liquor attained a level of 49.5% biodegradation by Day 28. This was equivalent to 39% biodegradation of the whole test material based on the initial DOC addition rate. The results are described in more detail in the Robust Summaries.

C. Ecotoxicity Data

Data on the SIDS ecotoxicity endpoints (acute toxicity to fish, daphnia and algae were conducted on spent pulping liquor. Because each of the ecotoxicity endpoints must be tested within a narrow pH range (6.5 to 8.5), consistent with maintaining the viability of the test organisms, it was necessary to neutralize the test substance (with a pH of approximately 11.5 to 13.5) before these tests were conducted. For all three tests, adjustment of the pH was carried out in the testing medium. The ecotoxicity data are summarized below.

Chemical	**Fish** **96 hr. LL_{50}**	**Daphnia** **48 hr. EL_{50}**	**Algae** **72 hr. $NOEL_r$**
Spent pulping liquor	>1000 mg/l	>1000 mg/l	5 mg/l

With respect to the results in algae, the 72 hr E_rC_{50} (Average specific growth rate) was estimated as 142.98 mg/l with a 72 h E_bC_{50} (AUC) estimated as 312.44 mg/l with a corresponding No Observed Effect Loading Rate ($NOEL_r$) of 5 mg/l for AUC and Average Specific Growth Rate. While the AUC is typically the most sensitive measure, in this case it was the growth rate. This is likely an effect of the dark color of the diluted test item which limited the amount of light and therefore the growth potential of the algae rather than true toxicity. Even though the relevance of these results is likely limited with respect to the potential ecotoxicity of spent pulping liquor *per se*, this is not an anticipated exposure scenario. However, these results are judged to be representative of what might occur if spent pulping liquor were to spill into an aquatic environment since dilution to a lower pH would also occur. These data are presented in greater detail in the Robust Summaries.

D. Human Health Effects

Data on the SIDS human health effects endpoints include acute toxicity, genotoxicity, repeat dose toxicity, reproductive and developmental toxicity. However, with the exception of the *in vitro* genotoxicity testing, all of the other human health effects endpoints require the test substance to be administered to animals either by gavage or in the diet. The high pH of the test material in this case would result in immediate corrosive effects in the animals. Not only would useful mammalian toxicity data not be obtained, but the spirit of the HPV program requires that testing in which animals would suffer should not be conducted. Therefore, AF&PA limited potential human health effects testing to the two genotoxicity endpoints (i.e., mutations in *Salmonella* and chromosomal aberrations in Chinese Hamster ovary cells) and did not perform any mammalian toxicity tests for spent pulping liquor. The rational for this decision is described below.

1. Likely Corrosive Effects

Given the extremely high pH of spent pulping liquor (approximately 11.5-13.5), it would be impossible to administer such a caustic material to test animals without causing them to suffer. It is well established that highly alkaline material can cause chemical burns. *"Extremely corrosive and reactive chemicals may produce immediate coagulative necrosis that results in substantial tissue damage. . . ."* (Casarett & Doull 1997) As a leading occupational medicine text notes: *"Alkalis not only coagulate tissue protein by dessication or salt formation, but they also saponify fats and cause liquefaction necrosis."* (Zenz 1994) The severity of the effect will depend on the corrosiveness of the chemical. (Olishifski 1974). OECD's Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation provides that *"If something is known to cause suffering in humans, it should be assumed to cause suffering in animals."* (OECD 2000).

With a pH in the range of 11.5 to 13.5 (and with the strong black liquor test material generally at the higher end of this range), spent pulping liquor is clearly corrosive. When shipped, spent pulping liquor is labeled as corrosive (UN1760 label) under Department of Transportation regulations. Manufacturers of spent pulping liquor comply with OSHA's Hazard Communication Standard, including providing Material Safety Data Sheets for the material. (OSHA defines as corrosive and therefore hazardous those chemicals that cause visible destruction of tissue at the site of contract. (Code of Federal Regulations, OSHA).) EPA automatically defines waste as hazardous due to the characteristic of corrosivity if the pH of the material is 12.5 or higher. (Code of Federal Regulations, EPA).

Thus, based on well-known characteristics of any corrosive material, one would expect spent pulping liquor to result in chemical burns. Whether by gavage or via administration in the diet, the high pH of spent pulping liquor would cause severe ulcerations or necrosis at the point of contact, i.e., esophagus or gastric mucosa, when fed to test animals.

2. Pertinent OECD Testing Guidelines

OECD guidelines provide that testing not be carried out when it will cause distress to the animals based on corrosive effects of the test substance. As noted in the guidelines for acute toxicity testing (OECD 401*), "Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out."* Indeed, OECD is currently taking steps to eliminate acute (LD_{50}) testing in light of animal use concerns. OECD 420 on acute toxicity further notes that*, "doses that are known to cause marked pain and distress, due to corrosive or severely irritant actions, need not be administered."* Moreover, OECD 422 governing repeat-dose testing states that, *"The highest dose level should be chosen with the aim of inducing toxic effects <u>but not</u> death nor <u>obvious suffering</u>."* [Emphasis added] It is not likely that these dual requirements could be satisfied simultaneously with such a corrosive material. For spent pulping liquor, even small doses would likely result in *"obvious suffering"* of the test animals.

3. Animal Welfare Act and Other Licensing Provisions

The Animal Welfare Act, 7 U.S.C. § 2131, requires that the Secretary of Agriculture set standards governing the humane handling, care, treatment, and transportation of animals by research facilities. The standards should ensure that experimental *procedures "ensure that animal pain and distress are minimized,"* and that the investigator considers *"alternatives to any procedure likely to produce pain to or distress in an experimental animal."*
The regulations are found at 9 C.F.R. Ch. 1. Generally, they require each research facility to ensure that its activities *"avoid or minimize discomfort, distress, and pain to the animals."* 9 C.F.R. § 2.31(d)(i). In its annual report, the research facility must certify that each principal investigator has considered alternatives to *"painful procedures,"* (9 C.F.R. § 2.36), defined as *"any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures."* 9 C.F.R. § 1.1 The Animal Welfare Act thus requires that testing that inflicts pain on the animals is to be carefully scrutinized.

4. Evaluation of Test Feasibility

Applying OECD guidelines and observing relevant provisions for animal welfare, it was inappropriate to conduct any animal testing of spent pulping liquor. At the pH of this material, primary toxicity is related to the inherent corrosivity of the material. Some of the pertinent OECD test guidelines allow for dilution of the test material used for animal testing. Based on the widely understood properties of corrosive material, it is expected that testing even of diluted spent pulping liquor would still produce unacceptable pain to the test species at doses high enough to produce valid toxicity tests. More importantly, in the case of spent pulping liquor, dilution would likely alter the composition of the material. Thus, testing a dilute substance would be addressing a different material - both in composition and because the fundamental corrosive property of the material would be changed.

5. EPA Guidance

The latest guidance from EPA (2000) states: *"In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested."* Given the high pH and corrosivity of this complex mixture, a thoughtful analysis of this issue led to the conclusion that mammalian testing of spent pulping liquor could not be justified.

6. *In Vitro* Genotoxicity Testing

Genetic testing is conducted to determine the effects of substances on genetic material (i.e., DNA and chromosomes). Genetic mutations are commonly measured in bacterial and mammalian cells, and the HPV program calls for completing both types of tests. The potential for spent pulping liquor to cause genotoxicity was tested in *Salmonella* to detect potential mutagenic effects and in Chinese Hamster ovary (CHO) cells to detect potential effects on chromosomal aberrations. In both tests, the pH of the test material was adjusted in order to ensure survival of the test organisms. The genotoxicity data are summarized below.

Chemical	**Ames *Salmonella***		**Chromosomal Aberration**	
	+S9	**-S9**	**+S9**	**-S9**
Spent pulping Liguor	Neg.	Neg.	Clastogenic (at overtly toxic concentrations)	Clastogenic

These data are presented in greater detail in the Robust Summaries.

IV. Overall Hazard Evaluation and Potential Exposure

For potential human health effects, the data on spent pulping liquor indicate that it is toxic (i.e., corrosive) due to its pH of 11.5 to13.5. Genotoxicity test results show no evidence of mutagenicity in *Salmonella* (i.e., Ames test) for spent pulping liquor. Chromosomal aberrations in Chinese hamster ovary (CHO) cells were evident only at concentrations of spent pulping liquor that were overtly toxic to the cells. Consequently, the only adverse health consequence that would be associated with any anticipated exposures to spent pulping liquor would be due to its corrosive nature. For potential ecotoxicological effects, the data on spent pulping liquor demonstrate that following neutralization (which would occur following dilution in the event of a spill) this substance would be non-toxic to aquatic organisms including fish (96 hr. LL_{50} > 1000 mg/l) and daphnia (48 hr. EL_{50} > 1000 mg/l), and essentially non-toxic to algae (72 hr. EL_{50} =312 mg/l).

With respect to potential exposure to spent pulping liquor, it is important to note that it is a byproduct that is almost always recycled at the production site and has only limited commercial use. Almost all of the spent pulping liquor generated is recycled for chemical and energy recovery. Controlled management of the material minimizes any potential for human or environmental exposure. An extremely small percentage of spent pulping liquor is transferred via pipeline on site to adjacent plants for lignin extraction and dimethyl sulfide extraction. Remaining spent pulping liquor is then returned to the pulp mill to be recycled for chemical and energy recovery. Strong black liquor (i.e., spent pulping liquor with some water removed) may be transported by truck to other pulping facilities that use it for chemical and energy recovery. In the event of an accidental spill into a body of water, pH effects are likely until sufficient dilution had occurred.

References

Adams, T.N., Frederick, W.J., Grace, T.M., Hupa, M., Lisa, K., Jones, A.K., and Tran, H. 1997. Kraft Recovery Boilers, T.N. Adams, Ed. TAPPI Press, Technology Park, P.O. Box 105113, Atlanta, GA 30348.

American Conference of Governmental Industrial Hygienists (ACGIH) 1991. Documentation of the Threshold Limit Values and Biological Exposure Indices, Sixth Edition. Cincinnati, OH.

Casarett & Doull's Toxicology, The Basic Science of Poisons, Klaassen, C. ed. 5th ed. 1996. p 533.

Code of Federal Regulations, OSHA, volume 29, Part 1910.1200, Appendix A, Health Hazard Determinations.

Code of Federal Regulations, EPA, volume 40, Part 261.22, Characteristic of Corrosivity.

EPA. Oct. 14, 2000. Letter from Susan Wayland, Deputy Assistant Administrator describing guidelines for limiting the unnecessary use of animals in implementing the HPV Challenge program.

Material Safety Data Sheets from various companies.

Olishifski, J, ed. Fundamentals of Industrial Hygiene (National Safety Council). 1974.

Organisation for Economic Co-operation and Development (OECD). 2000. Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Environment Directorate Joint Meeting of The Chemicals Committee and the Working Party On Chemicals, Pesticides And Biotechnology. Env/JM/Mono(2000)7.

OECD Guideline For Testing of Chemicals, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, OECD 422.

OECD Guideline For Testing of Chemicals, Acute Oral Toxicity, OECD 401.

OECD Guideline For Testing of Chemicals, Acute Oral Toxicity - Fixed Dose Method, OECD 420.

Venkatesh, V. and Nguyen, X.N. 1985. Chemical Recovery in the Alkaline Pulping Process, G. Hough, Ed., TAPPI Press Technology Park, P.O. Box 105113, Atlanta, GA 30348. Chapter 3. Evaporation of Black Liquor, pp 15-85.

Zenz, C, Dickerson, O., and Horvath, E., Eds. Occupational Medicine, 3d edition (1994).